Pharm. Sci. 2000, 21, 45). Examples of these transduction signals are derived from viral coat proteins such as Tat from HIV and VP22 from herpes simplex virus, and a transcriptional factor from *Drosophila*, ANTP. The peptides Tat (Tyr-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg, SEQ ID 1), VP22 (Asp-Ala-Ala-Thr-Ala-Thr-Arg-Gly-Arg-Ser-Ala-Ala-Ser-Arg-Pro-Thr-Glu-Arg-Pro-Arg-Ala-Pro-Ala-Arg-Ser-Ala-Ser-Arg-Pro-Arg-Arg-Pro-Val-Glu, SEQ ID 2), and ANTP (Arg-Gln-Iso-Lys-Iso-Trp-Phe-Gln-Asn-Arg-Arg-Met-Lys-Trp-Lys-Lys, SEQ ID 3) share no sequence motif other than number of cationic (lysine and arginine) residues. In addition, reports of synthetic peptides possessing no homology other than a propensity of cationic charge (net overall cationic charge) have also been shown to posses transduction activity (Service, R.F. Science 2000, 288, 28.)

Nuclear localizing signals enhance the targeting of the gene into proximity of the nucleus and/or its entry into the nucleus. Such nuclear transport signals can be a protein or a peptide such as the SV40 large T ag NLS or the nucleoplasmin NLS. These nuclear localizing signals interact with a variety of nuclear transport factors such as the NLS receptor (karyopherin alpha) which then interacts with karyopherin beta. The nuclear transport proteins themselves could also function as NLS's since they are targeted to the nuclear pore and nucleus.

Signals that enhance release from intracellular compartments (releasing signals) can cause DNA release from intracellular compartments such as endosomes (early and late), lysosomes, phagosomes, vesicle, endoplasmic reticulum, golgi apparatus, trans golgi network (TGN), and sarcoplasmic reticulum. Release includes movement out of an intracellular compartment into cytoplasm or into an organelle such as the nucleus. Releasing signals include chemicals such as chloroquine, bafilomycin or Brefeldin A1 and the ER-retaining signal (KDEL sequence), viral components such as influenza virus hemagglutinin subunit HA-2 peptides and other types of amphipathic peptides. Cellular receptor signals are any signal that enhances the association of the gene or particle with a

2,3-dicarboximide esters, p-nitrophenyl esters, pentafluorophenyl esters, 4-dimethylaminopyridinium amides, and acyl imidazoles.

A nucleophile is a species possessing one or more electron-rich sites, such as an unshared pair of electrons, the negative end of a polar bond, or pi electrons.

Examples

Example 1: Synthesis of Cysteine-Terminal Tat Peptide (Tat-Cys).

Peptide syntheses were performed using standard solid phase peptide techniques using FMOC chemistry. A cysteine was added to the amino terminus of Tat to allow for conjugation through the thiol group to make the peptide Tyr-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg-Cys (Tat-Cys, SEQ ID 4).

Example 2: Synthesis of noncleavably linked (irreversible covalent) Tat-Cys and fluorescein through a thioether bond.

To a solution of succinimidyl-4-(N-maleimidomethyl) cyclohexane-carboxylate (SMCC from Pierce) 1.0 mg in 0.1 mL dimethylformamide was added 1.2 mg (1 eq) of 4'-(aminomethyl)fluorescein. After two hours, this solution was added to a 1 mL aqueous solution of 8.4 mg Tat-Cys (1 eq). The solution was buffered to pH 8 by the addition of potassium carbonate. This solution was used for transport studies without further purification.

H_2N-EEEEEEEE-$NHCH_2CH_2NH_2$ (SEQ ID 5) (5.0 mg, 0.0052 mmol, Genosis) was taken up in 0.1 mL HEPES (250 mM, pH 7.5). 5,5'-dithiobis[succinimidyl(2-nitrobenzoate)] (3.1 mg, 0.0052) was added with 0.2 mL DMSO and the mixture was stirred overnight at room temperature. After 16 hr the solution was heated to 70°C for 10 min, cooled to room temperature and diluted to 1.10 mL with DMSO.

Example 47:Complex Formation with 5,5'-Dithiobis(2-nitrobenzoic acid) – Poly-Glutamicacid (8mer) Copolymer

Fluorescein labeled DNA was used for the determination of DNA condensation in complexes with 5,5'-Dithiobis(2-nitrobenzoic acid) – Poly-Glutamicacid (8mer) Copolymer. pDNA was modified to a level of 1 fluorescein per 20 bases using Mirus' *LabelIT*™ Fluorescein kit. The fluorescence was determined using a fluorescence spectrophotometer (Shimadzo RF-1501 Fluorescence Spectrophotometer), at an excitation wavelength of 497 nm, and an emission wavelength of 520 nm.

To fluorescein labeled DNA (10 μg) in 1 mL HEPES (25 mM, pH 7.5) was added polyornithine (18 μg, Sigma Chemical Company). The mixtures were held at room temperature for 5 minutes and the fluorescence was determined. (see: Trubetskoy, V. S., Slattum, P. M., Hagstrom, J. E., Wolff, J. A., Budker, V. G., "Quantitative Assessment of DNA Condensation," Anal. Biochem (1999) incorporated by reference) Since fluorescence intensity is decreased by DNA condensation, results indicate that polyornithine compacts DNA. To the resulting complex was added 5,5'-Dithiobis(2-nitrobenzoic acid) – Poly-Glutamicacid (8mer) Copolymer (60 μg), and the fluorescence was again determined. The fluorescence of the sample decreased further.

Upon the addition of 5,5'-Dithiobis(2-nitrobenzoic acid) – Poly-Glutamicacid (8mer) Copolymer to the sample, the fluorescence decreased, indicating the formation a triple complex. No competition of the 5,5'-Dithiobis(2-nitrobenzoic acid) – Poly-Glutamicacid (8mer) Copolymer for the polyornithine was observed (increase in fluorescence).